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Presentation of Benzo(a)pyrene to Microsomal Enzymes by Asbestos Fibers in the Salmonella/Mammalian Microsome Mutagenicity Test

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The potential effect of asbestos fibers on mutagenicity of benzo(a)pyrene was investigated by using the Ames test.

Asbestos fibers without a coating of benzo(a)pyrene or benzo(a)pyrene when not dissolved in DMSO lacked any mutagenic effect in the Salmonella/mammalian microsome mutagenicity test. However, when benzo(a)pyrene was adsorbed onto asbestos, significant numbers of mutated bacteria were observed. This shows that asbestos fibers can serve a carrier role presenting benzo(a)pyrene to the enzymatic microsomal system, thus enhancing mutagenicity of this compound.

Introduction

Epidemiological and experimental animal studies have documented the carcinogenic effect of asbestos (*1-5*). Little is known, however, about the mechanism of asbestos carcinogenicity. The bacterial mutagenicity test (*6*) and sister chromatid exchange methods (*7, 8*) did not detect any mutagenic activity of asbestos fibers. Also, no direct evidence of mutagenic effect of asbestos could be derived from the studies of chromosome aberrations. Indeed, some chromosome aberrations were found (*9*); however, this may reflect the cytotoxic effect without further genetic significance. All these data suggest that asbestos fibers do not act primarily as genotoxic carcinogens inducing alteration in DNA but rather play the role of cofactor. In favor of this hypothesis are recent papers showing an enhancing effect of asbestos fibers on solubilization and dispersion of benzo(a)pyrene (BaP) in the lipid bilayer of the cellular membrane (*10, 11*). However, there are no data on the biological significance of the presentation of BaP by asbestos fibers to microsome membranes. This problem was investigated with the use of the Salmonella/mammalian microsome mutagenicity test.

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Materials and Methods

Chemicals

Benzo(a)pyrene (BaP), DMSO, NADP and glucose-6-phosphate were purchased from Sigma Chem. Co., UICC asbestos samples (amosite, crocidolite and chrysotile) were generously supplied by Dr. V. Timbrell.

Aqueous Suspension of Asbestos Fibers

The aqueous suspension of asbestos fibers was prepared by mixing asbestos samples with M-9 buffer (0.06 *M* phosphate, 0.1 *M* NaCl), pH 7, and sonicated at 20 kHz for 10 min in a bath-type sonicator.

Aqueous Microcrystalline Suspension of BaP

The aqueous microcrystalline suspension of BaP was prepared by evaporation of a benzene solution of BaP to dryness, addition of M-9 buffer and sonicated as above.

Adsorption of BaP onto Asbestos Fibers

Method of Lakowicz and Hylden. In this method (*12*), 1 g asbestos fibers was mixed with 0.3 mg of BaP dissolved in 10 mL of benzene. The solvent was then evaporated under reduced pressure at 70°C.

During this procedure the suspension was constantly agitated. After the samples appeared dry, they were placed under a vacuum for 30-45 min at 7°C. Next, the buffer (0.06 *M* phosphate, 0.1 *M* NaCl, pH 7.0), was added and the suspension was sonicated for 10 min at 20 kHz. The samples were ready for further use in the *S. typhimurium* system.

Method of Kandaswami and O'Brien. In this method (*13*), the aqueous suspension of microcrystalline benzo(a)pyrene and the aqueous suspension of asbestos fibers after sonication were mixed and incubated for 10 min at 37°C. The asbestos to BaP ratio per dry weight was 1:0.0003.

Preparation of Microsomal Enzymes

The liver homogenate fraction (S-9) was prepared from Aroclor 1254-treated male Wistar rats. The S-9 fraction was supplemented with cofactors according to the method of Ames (*14*).

Bacterial Strains

The histidine auxotrophs (*his*$^-$) of *S. typhimurium* TA 98 and TA 100 strains, carrying the plasmid pKM 101, were generously provided by Dr. B. N. Ames.

Mutagenic Assay

The Salmonella/microsome mutagenicity test was performed according to the method of Ames (*14*). Experiments were designed as follows. The mutagenicity of BaP dissolved in DMSO was established as a positive control. The mutagenicity of asbestos fibers alone was checked to evaluate its intrinsic mutagenic potency. Also, the mutagenicity of microcrystalline BaP neither dissolved in DMSO nor adsorbed onto asbestos fibers (but just added) was investigated in order to obtain a reference basis for the results of experiments employing BaP adsorbed onto asbestos fibers. Finally, BaP adsorbed onto asbestos fibers was evaluated as a potential mutagenic substance in a medium lacking DMSO.

Results and Discussion

Asbestos fibers did not induce any significant increase in the number of mutated bacteria in the *S. typhimurium* system. The inability of asbestos fibers to induce mutations was seen in the dose range from 1 to 20 mg/plate, independent of the use of microsomal fraction S-9. Our results corroborate the findings of Chamberlain and Tarmy (*6*).

In the preliminary experiments the mutagenic potential of BaP dissolved in DMSO was compared with the results obtained when BaP was adsorbed onto asbestos by using the described methods above (*12, 13*). As could be predicted, the DMSO solution of BaP exerted significant mutagenicity in the system employing *S. typhimurium* TA 100 strain (Table 1). Such mutagenicity was not seen when a BaP microcrystalline suspension lacking DMSO was used (Table 1). During the same experiments asbestos fibers coated with BaP were exposed to the *S. typhimurium* TA 100 strain. The presence of asbestos fibers coated with BaP according to the method of Lakowicz and Hylden (*12*) resulted in a significant increase in the number of revertants when 5 and 10 μg of BaP adsorbed onto asbestos were used (Table 1). The increase in the num-

Table 1. Mutagenicity exerted by asbestos fibers coated with BaP according to the methods of Lakowicz and Hylden, and Kandaswami and O'Brien with the use of *S. typhimurium* strain TA 100 as target cells.

Asbestos (number of experiments)	Method of BaP adsorption	The number of *his*$^+$ revertants (average ± SE) with various BaP doses			Correlation coefficient
		0	5 μg/plate	10 μg/plate	*r*
Amosite (*N* = 3)	Lakowicz and Hylden	194 ± 10	582 ± 21	968 ± 75	0.966
	Kandaswami and O'Brien	194 ± 10	195 ± 11	175 ± 9	− 0.315
	No adsorption[a]	194 ± 10	179 ± 8	193 ± 15	− 0.090
Chrysotile (*N* = 2)	Lakowicz and Hylden	195 ± 12	657 ± 25	967 ± 48	0.979
	Kandaswami and O'Brien	195 ± 12	195 ± 10	215 ± 8	0.388
	No adsorption[a]	195 ± 12	212 ± 9	234 ± 10	0.348
Crocidolite (*N* = 2)	Lakowicz and Hylden	216 ± 8	803 ± 80	1143 ± 233	0.820
	Kandaswami and O'Brien	216 ± 8	208 ± 13	210 ± 20	− 0.099
	No adsorption[a]	216 ± 8	234 ± 10	247 ± 7	0.725
None (*N* = 3)	BaP-DMSO solution without asbestos	201 ± 10	603 ± 44	1051 ± 122	0.920
	Aqueous suspension of BaP without asbestos	201 ± 10	208 ± 6	236 ± 16	0.390

[a]Asbestos and BaP were added to the system without prior adsorption procedure.

ber of revertants was comparable to that obtained when the same doses of BaP dissolved in DMSO were added to the system instead of BaP adsorbed onto asbestos. Also a very similar increase was observed independent of the type of asbestos used (amosite, chrysotile, crocidolite) (Table 1).

In parallel experiments, asbestos fibers coated with BaP by the Kandaswami and O'Brien method (*13*) did not show any mutagenicity (Table 1).

In further experiments, only the Lakowicz and Hylden method (*12*) of adsorption was used. In these experiments the dose-response relationship for mutation induced by BaP adsorbed on asbestos was investigated in the range of 1.5 and 6.0 μg. TA 98 and

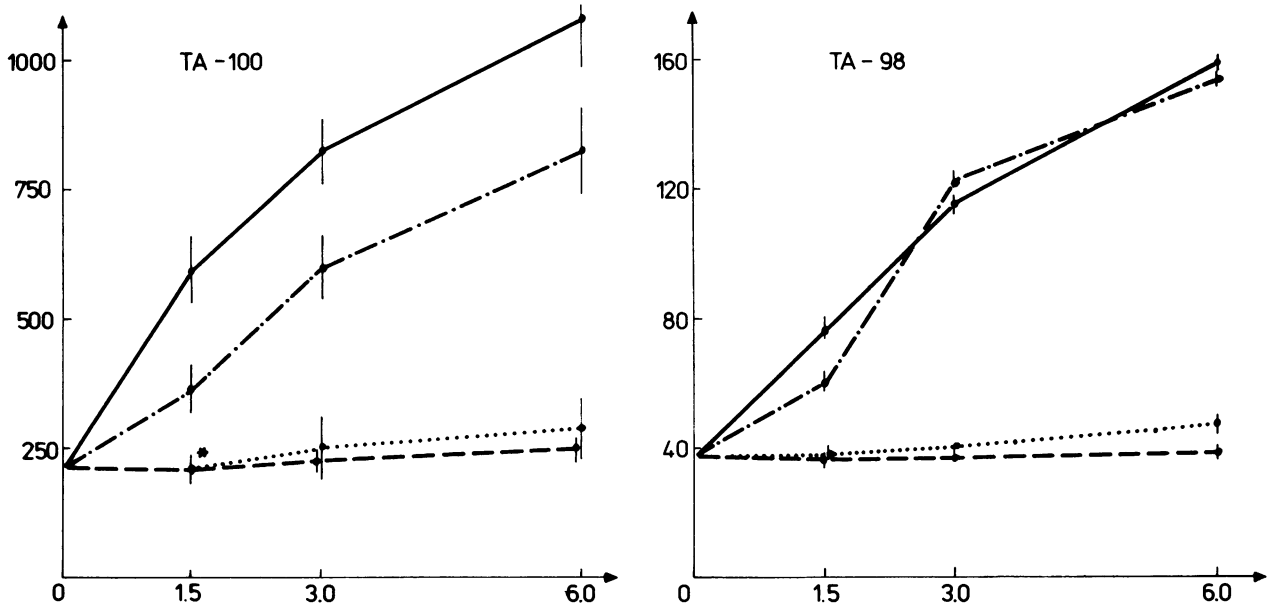

FIGURE 1. Mutagenicity of amosite fibers coated with BaP in the *S. typhimurium*/microsome system: (- -) microcrystalline BaP; (· · ·) microcrystalline BaP mixed with the fibers; (— · —) BaP dissolved in DMSO; (——)BaP adsorbed on the fibers. Each point represents the mean value of three independent experiments ± SE. Abscissa: μg of BaP per plate; ordinate: number of revertant colonies per plate.

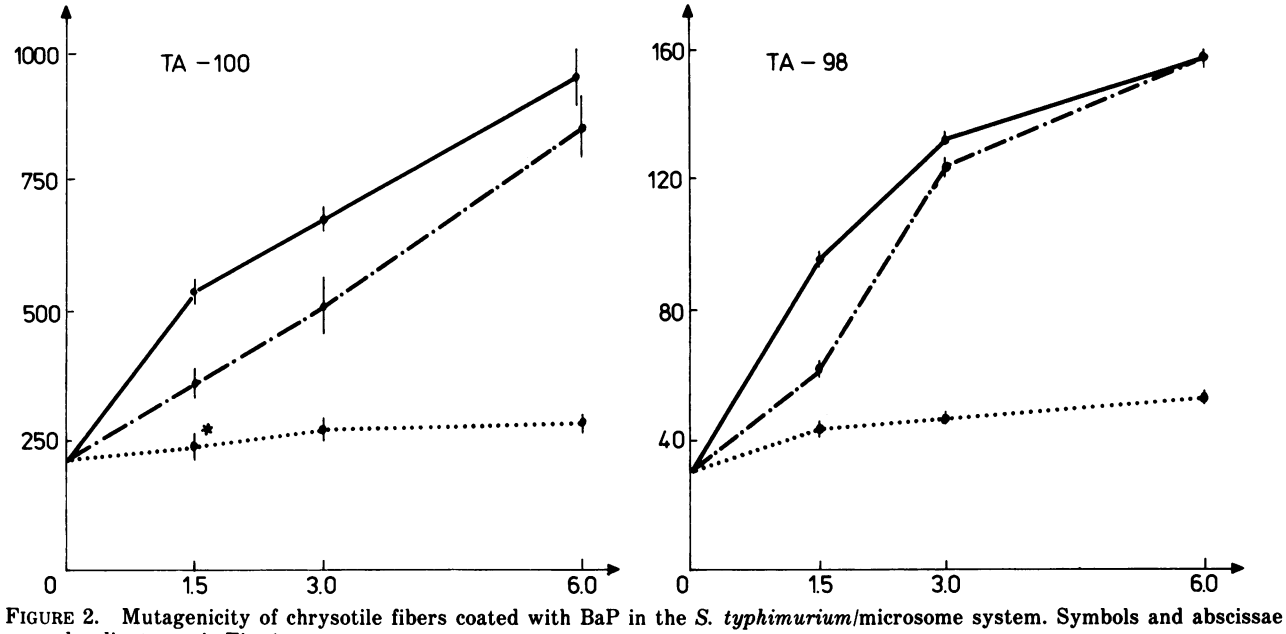

FIGURE 2. Mutagenicity of chrysotile fibers coated with BaP in the *S. typhimurium*/microsome system. Symbols and abscissae and ordinates as in Fig. 1

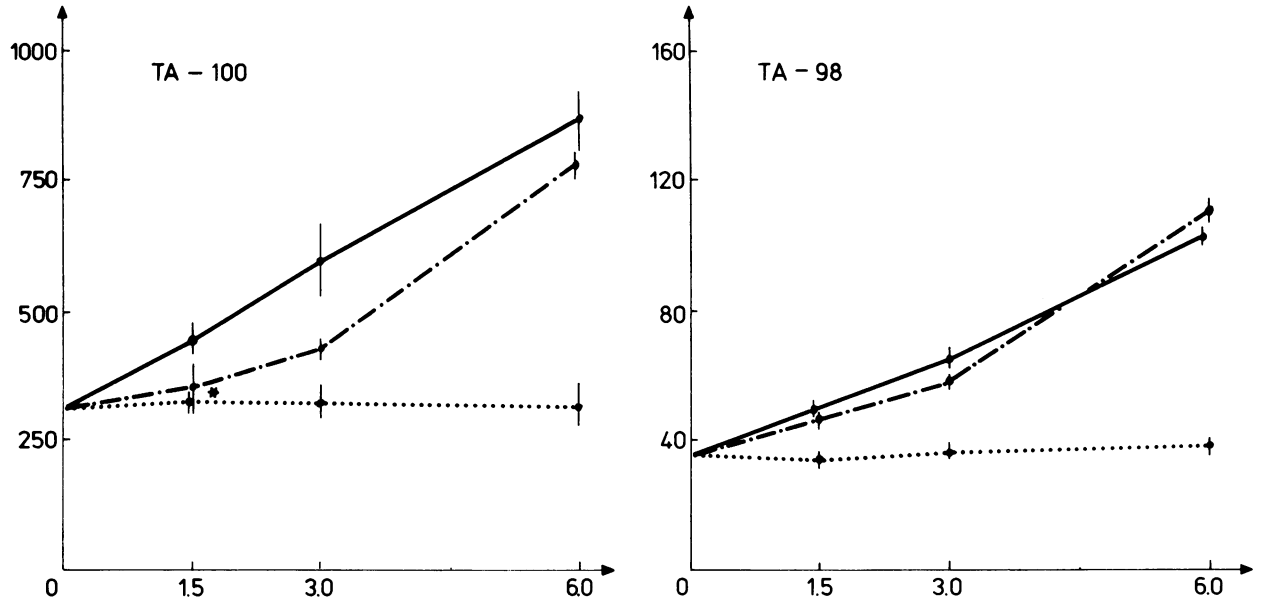

FIGURE 3. Mutagenicity of crocidolite fibers coated with BaP in the *S. typhimurium*/microsome system. Symbols and abscissae and ordinates as in Fig. 1.

TA 100 strains were used. Again, it was seen that BaP, when adsorbed onto fibers, exerted a significant mutagenic effect in spite of the absence of DMSO as a solvent (Figs. 1, 2 and 3). A significant increase in the number of revertants was seen at the 1.5 μg BaP dose. This increase was seen with all three types of asbestos used. Also very similar results were obtained with both *S. typhimurium* strains employed (TA 98, TA 100) (Figs. 1-3).

Furthermore, a significant correlation of coefficients was found between the dose of BaP adsorbed onto asbestos and the number of mutated bacteria. The correlation coefficients were: 0.96, 0.93 and 0.94 for chrysotile, amosite and crocidolite fibers, respectively, with strain TA 100. The analogous figures for TA 98 strain were: 0.91, 0.81 and 0.94. These results showed that BaP adsorbed onto asbestos fibers is readily accessible to the microsome system independent of the presence of DMSO.

Our results are easily understandable in the light of work done by Lakowicz et al. (*10-12*), which showed that adsorption of BaP onto asbestos or other particles increases membrane uptake of BaP. This was not related to the specific minerological character of different types of asbestos (*10*). Amosite, crocidolite and chrysotile were equally effective in BaP presentation (Figs. 1-3).

BaP asbestos fibers adsorbed by the method of Lakowicz and Hylden showed mutagenic activity (*12*). In this method BaP dissolved in benzene is mixed with asbestos fibers and then the solvent is evaporated at 70°C under vacuum. A much simpler procedure was proposed by Kandaswami and O'Brien (*13*). Asbestos fibers are mixed with an aqueous microcrystalline suspension of BaP at 37°C. BaP adsorbed onto asbestos under these conditions did not show any mutagenic potential in the Ames test (Table 1).

It is known from the animal studies that the instillation of particles with adsorbed polynuclear aromatic hydrocarbons produces greater tumorigenesis than the instillation of particles alone or carcinogen separately (*15*). Our results and those of Lakowicz et al. (*10-12*) showed that chemical carcinogen is more readily accessible to the microsomal enzymes when it is presented as a compound adsorbed onto the particles, and we found that this phenomenon has a biological value at least in the Ames test. In view of data presented above, the amplifying effect of asbestos exposure and cigarette smoking on the incidence of lung cancer (*16*) is clearly understandable.

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